Docket No. 2026-4202US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Frank CUTTITTA, Alfredo MARTINEZ, Mae Jean MILLER, Edward J. UNSWORTH, William HOOK, Thomas WALSH, Karen GRAY and Charles MACRI	Group Art Unit:	To Be Assigned
Serial No.:	To Be Assigned (Divisional of 09/011,922)	Examiner:	To Be Assigned
Filed:	Herewith (August 16, 2001)		
For:	FUNCTIONAL ROLE OF ADRENOMEDULLIN (AM) AND THE GENE-RELATED PRODUCT (PAMP) IN HUMAN PATHOLOGY AND PHYSIOLOGY		

EXPRESS MAIL CERTIFICATE

Express Mail Label No.: **EL 853 256 591 US**

Date of Deposit: **August 16, 2001**

I hereby certify that the following attached paper(s) and/or fee

1. Divisional patent application containing 77 total pages, including 73 pages of Specification, 3 pages of Claims, 1 page of Abstract;
2. 26 Sheets of Drawings;
3. Application Transmittal (5 pages);
4. Application Fee (check for $710.00);
5. Preliminary Amendment (12 pages);
6. Executed Declaration and Power of Attorney as filed in parent application (4 pages);
7. Sequence Listing (6 pages);
8. Computer-readable form of Sequence Listing (3.5 inch disk);
9. Statement of Identity for Sequence Listing (1 page);
10. Copy of Recorded Assignment from parent application (10 pages);
11 Copy of International Search Report (3 pages);
12. Copy of PCT Written Opinion (6 pages);
13. Copy of Reply to PCT Written Opinion (PCT/US96/13286) including replacement pages of specification and replacement claims (25 pages);
14. Information Disclosure Statement and PTO 1449 Form as filed in parent application (7 pages);
15. Return Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to: **BOX: PATENT APPLICATION**, Commissioner for Patents, Washington, D.C. 20231.

Jesus Raul Remedios
(Typed or printed name of person mailing papers(s) and/or fee)

(Signature of person mailing paper(s) and/or fee)

Correspondence Address:

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08/17/01

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Docket No. 2026-4202US4

Express Mail No. EL 853 256 591 US

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PATENT TRADEMARK OFFICE

↑AFFIX CUSTOMER NO. LABEL ABOVE ↑

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND FEE TRANSMITTAL §(1.53(b))

Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s) names and addresses:

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Bethesda, Maryland 20817 USA

(7) Karen GRAY
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Gaithersburg, Maryland 20879 USA

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3302 Saul Road
Kensington, Maryland 20895 USA

For: **FUNCTIONAL ROLE OF ADRENOMEDULLIN (AM) AND THE GENE-RELATED PRODUCT (PAMP) IN HUMAN PATHOLOGY AND PHYSIOLOGY**

Enclosed Are:

73 page(s) of Specification
3 page(s) of Claims
1 page(s) of Abstract
77 Total page(s) of application, including 73 pages of specification; 3 pages of claims; and 1 page of abstract (on PCT cover sheet)
26 sheets of drawings; (Use formal drawings from parent application.)
4 page(s) of Declaration and Power of Attorney

- ☐ Unsigned
- ☐ Newly Executed
- ☒ Copy from prior application
- ☐ Deletion of inventors including Signed Statement under 37 C.F.R. §1.63(d)(2)

☐ **REQUEST AND CERTIFICATION UNDER 35 U.S.C. §122(b)(2)(B)(i) (form PTO/SB/35)**
As indicated on the attached Request and Certification, Applicant(s) certify that the invention disclosed in the attached application HAS NOT and WILL NOT be the subject of an application filed in another country, or under a multilateral agreement, that requires publication at eighteen months after filing. Applicant(s) therefore request(s) that the attached application NOT be published under 35 U.S.C. §122(b).

☒ Incorporation by Reference:

☒ The entire disclosure of the prior application, from which a copy of the combined Declaration and Power of Attorney is supplied herein, is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.

☐ Deletion of Inventors (37 C.F.R. §1.63(d) and §1.33(b)

Signed statement attached deleting inventor(s) named in the prior application serial no. ____, filed _____.

☐ Microfiche Computer Program (Appendix)

☒ 6 Page(s) of Sequence Listing

☒ Computer readable disk containing Sequence Listing

☒ 1 Page, Statement under 37 C.F.R. §1.821(f) that computer and paper copies of the Sequence Listing are the same.

☒ Assignment Papers (assignment cover sheet and assignment documents)

- ☐ A check in the amount of $40.00 for recording the Assignment
- ☐ Charge the Assignment Recordation Fee to Deposit Account No. 13-4500, Order No. _____.
- ☒ Assignment Papers filed in the parent application Serial No. 09/011,922, filed February 17, 1998. (10 pages of copy of Assignment Papers as recorded in parent application on September 8, 1998.)

☐ Certification of chain of title pursuant to 37 C.F.R. §3.73(b)

☐ Priority is claimed under 35 U.S.C. §119 for:
Application No(s). _____ , filed _____, in _____ (country).

- ☐ Certified Copy of Priority Document(s) [_____]
  - ☐ filed herewith
  - ☐ filed in application Serial No. _____, filed _____.
- ☐ English translation document(s) [_____]
  - ☐ filed herewith
  - ☐ filed in application Serial No. _____, filed _____.

☒ Priority is claimed under 35 U.S.C. §119(e) for:
Provisional Application No. 60/002,514, filed August 18, 1995; Provisional Application No. 60/002,936, filed August 30, 1995; and Provisional Application No. 60/013,172, filed March 12, 1996.

☒ Information Disclosure Statement

- ☐ Copy of [_____] cited references
- ☒ PTO Form-1449
- ☒ IDS References provided in parent application Serial No. 09/011,922, filed February 17, 1998.

☐ Related Case Statement under 37 C.F.R. §1.98(a)(2)(iii)

- ☐ A copy of related pending U.S. Application(s) Serial No(s): _____, filed _____, respectively, is attached hereto.
- ☐ A copy of related pending U.S. Application(s) entitled, _____, filed _____ to inventor(s) _____, respectively, is attached hereto.
- ☐ A copy of each related application(s) was submitted in parent application serial no. ____, filed _____.

☒ Preliminary Amendment

☒ Return receipt postcard (MPEP 503)

☒ This is a ☐ continuation ☒ divisional ☐ continuation-in-part of prior application serial no. 09/011,922, filed February 17, 1998, to which priority under 35 U.S.C. §120 is claimed.

- ☐ Cancel in this application original claims _____ of the parent application before

calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☒ A Preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application).

☒ The status of the parent application is as follows:

☐ A Petition for Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until _____.

☐ A copy of the Petition for Extension of Time in the co-pending parent application is attached.

☒ No Petition for Extension of Time and Fee therefor are necessary in the co-pending parent application.

☐ Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.

☒ Transfer the drawing(s) from the parent application to this application

☒ Amend the specification by inserting before the first line the sentence:
This is ☐ continuation ☒ divisional ☐ continuation-in-part of co-pending application Serial No. 09/011,922, filed February 17, 1998.

I. CALCULATION OF APPLICATION FEE				
	Number Filed	Number Extra	Rate	Basic Fee $710.00/355.00
Total Claims	27- 20 =	7x	$18.00/ $9.00	$126.00
Independent Claims	11- 3 =	8x	$80.00/ $40.00	$640.00
☒ Multiple Dependent Claims		If marked, add fee of $270.00 ($135.00)		$270.00
			TOTAL:	$1746.00

☐ Small entity status is or has been claimed. Reduced fees under 37 C.F.R. §1.9 (f) paid herewith
$_____.

☒ A check in the amount of $710.00 in payment of the application filing fee is attached.

☐ Charge fee to Deposit Account No. 13-4500, Order No. _____. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required for filing this application pursuant to 37 CFR §1.16, **including all extension of time fees pursuant to 37 C.F.R. § 1.17 for maintaining copendency** with the parent

application, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2026-4202US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Dated: August 16, 2001

By: Leslie Serunian
Leslie A. Serunian
Registration No. 35,353

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PATENT
Docket No. 2026-4202 US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	F. CUTTITTA et al.	Group Art Unit: To Be Assigned
Serial No.	:	09/011,922	Examiner: To Be Assigned
Filed	:	February 17, 1998	
For	:	Functional Role of Adrenomedullin (AM) and its Gene-Related Product (PAMP) in Human Pathology and Physiology	

EXPRESS MAIL CERTIFICATE

Express Mail Label No. TB 608 954 896 US

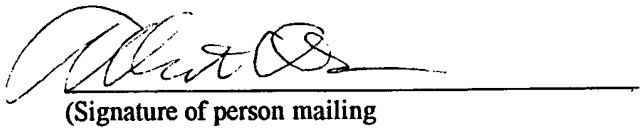
Date of Deposit September 4, 1998

I hereby certify that the following attached paper(s) and/or fee

1) Assignment Recordation Form Cover Sheet
2) Executed Assignment
3) Recordation Fee (check for $40.00)
4) Return Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to **BOX: ASSIGNMENT**, Assistant Commissioner for Patents, Washington, D.C. 20231.

Albert Isles
(Typed or printed name of person mailing paper(s) and/or fee)



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Docket No.: 2026-4202PCT

IN THE EUROPEAN PATENT OFFICE (IPEA)

Applicant : The Government of the United States of America, as represented by the Secretary, Department of Health and Human Services, et al.

International Application No.: PCT/US96/13286

International Filing Date : 16 August 1996 (16.08.1996)

For : **FUNCTIONAL ROLE OF ADRENOMEDULLIN (AM) AND THE GENE-RELATED PRODUCT (PAMP) IN HUMAN PATHOLOGY AND PHYSIOLOGY**

VIA FACSIMILE (011) 49-89-2399-4465
European Patent Office
IPEA
D-80298 Munich
Germany

Attn: Examiner A. Kronester-Frei

TRANSMITTAL FOR REPLY TO WRITTEN OPINION UNDER RULE 66.3

Sir:

Transmitted herewith is a Reply to the Written Opinion dated 23 May 1997 for the above-identified International Application. Also transmitted herewith are substitute claims 1-43 on substitute sheets 73/1, 73/2, 74/1, 74/2, 75/1 and 76-80, and substitute sheets 3/1, 3/2, 8/1, 12/1, 13/1 and 15/1 of the specification. It is respectfully submitted that this Reply is being timely filed by the specified due date of August 23, 1997.

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Dated: August 21, 1997

By: Leslie Serunian
Leslie A. Serunian
Agent for Applicant

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EL 853 256 591 US

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Docket No.: 2026-4202PC

IN THE EUROPEAN PATENT OFFICE (IPEA)

Applicant : The Government of the United States of America, as represented by the Secretary, Department of Health and Human Services, et al.

International Application No.: PCT/US96/13286

International Filing Date : 16 August 1996 (16.08.1996)

For : **FUNCTIONAL ROLE OF ADRENOMEDULLIN (AM) AND THE GENE-RELATED PRODUCT (PAMP) IN HUMAN PATHOLOGY AND PHYSIOLOGY**

VIA FACSIMILE (011) 49-89-2399-4465

European Patent Office
IPEA
D-80298 Munich
Germany

Attn: Examiner A. Kronester-Frei

REPLY TO WRITTEN OPINION UNDER RULE 66.3

Sir:

In accordance with Rule 66.3, applicants communicate this Reply to the Written Opinion dated 23 May 1997 for the above-identified International Application. It is respectfully submitted that this Reply is being timely filed on the date even herewith.

In the Claims:

Amended and new claims are provided as Claims 1-43 on replacement sheets

73/1 through 80, enclosed herewith.

Specifically, the claims on the replacement sheets differ from those on original sheets 73-75 as follows:

Replacement Sheet 73/1:

Replacement Claim 1: Original Claim 1 has been amended to delete "PAMP-20 (SEQ ID NO: 7)".

Replacement Claim 2: The subject matter of original Claim 2 remains the same.

Replacement Claims 3 and 4: Original Claim 3 has been rewritten as Claims 3 and 4, which are in a form acceptable for international examination.

Replacement Claim 5: Claim 5 contains the subject matter of Original Claim 4, with prostate and chondrosarcoma added, as supported by Table 3, page 52 of the original specification.

Replacement Claim 6: Claim 6 corresponds to Original Claim 5, and contains the further recitation that the disease being diagnosed or monitored is other than hypotension, hypertension or cardiac incompetence.

On Replacement Sheet 73/2:

Replacement Claim 7: Claim 7 corresponds to Original Claim 6 and contains the wording "hematopoietic cell disease" instead of "blood related diseases", as supported by the present specification at page 3, line 15.

Replacement Sheets 74/1-74/2:

Replacement Claims 8 and 9: Original Claim 7 has been replaced by Claims 8 and 9, which are in a form acceptable for international examination.

Replacement Claims 10 and 11: Original Claim 8 has been replaced by Claims 10 and 11, which are in a form acceptable for international examination.

Replacement Claim 12: Original Claim 9 has been replaced by Claim 12.

Replacement Claim 13: Original Claim 10 has been replaced by Claim 13, which is in a form acceptable for international examination.

Replacement Sheet 75/1:

Replacement Claim 14: Original Claim 10 has been replaced by Claim 14, which is in a form acceptable for international examination.

Replacement Claims 15 and 16: Original Claim 11 has been replaced by Claims 15 and 16, which are in a form acceptable for international examination. Support for the use of AM antibodies in regulating, lessening or inhibiting an inflammatory response by immune response cells such as macrophages can be found in the specification of the original application at Fig. 7A showing immunoreactivity of AM in alveolar macrophages and in Example 10 describing immunoreactivity analyses and the expression of AM mRNA in macrophages.

Replacement Sheets 76-80:

Replacement Claims 17 and 18: Original Claim 12 has been replaced by Claims 17 and 18, which are in a form acceptable for international examination.

Replacement Claims 19 and 20: Original Claim 13 has been replaced by Claims 19 and 20, which are in a form acceptable for international examination.

Replacement Claims 21 and 22: Original Claim 14 has been replaced by Claims 21 and 22, which are in a form acceptable for international examination.

Replacement Claim 23: Claim 23 contains the subject matter of Original Claim 15.

Replacement Claim 24: Claim 24 contains the subject matter of Original Claim 16.

Claims 25-42: Claims 25-42 cover uses of the isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7), as related to the subject matter described for AM peptides having SEQ ID NOS: 1-3 described in replacement Claims 3-22 .

Claim 43: Claim 43 covers isolated AM oligonucleotides as described in Claim 1 as originally filed.

Support for the replacement claims:

Support for the replacement claims is found in the claims as originally filed. Replacement claims 3-5, 8-22, and 25-42 have been presented to describe the adrenomedullin peptides of the present invention for first use in a medical treatment and in the manufacture of a medicament for a new medical treatment.

Reply to The Reasoned Statement Under Rule 66.2(a)(ii)

The Examining Authority has reasoned that D1 discloses in Example 7 isolated peptides, i.e., (-73)-(-54) and (-61)-(-54), used as an antigen and in the production of an antibody against pro-AM-N20, so as to cause SEQ ID NO: 7 according to claim 1 to lack novelty.

Applicants have submitted herewith replacement claims which describe the invention of the present application. Replacement Claims 1 and 2 recite applicants' novel isolated adrenomedullin (AM) peptides and antibodies reactive therewith, respectively. The claimed AM peptides and antibodies and their newly-discovered uses are neither taught nor suggested by the cited prior art.

D1 provides the sequence of a large AM peptide and does not disclose or recognize the particular isolated AM peptides of the present invention. D1 does not contemplate the uses discovered by applicants for the claimed AM peptides, or antibodies reactive therewith. Further, D1 fails to teach or recognize applicants' PAMP-20 peptide having SEQ ID NO: 7 in the novel and inventive medical uses and in the manufacture of a medicament as presently claimed.

Applicants have newly discovered that the isolated AM peptides as specified, or antibodies reactive therewith, are able to be utilized for treating, diagnosing or monitoring diseases other than those related to general vasoreactive symptoms such as hypertension, hypotension or cardiac incompetence. D1 is silent regarding the use of applicants' isolated AM peptides in a number of newly-discovered medical applications: for example, to regulate insulin secretion and blood glucose metabolism; to diagnose and treat pregnancy-related conditions, such as preeclampsia, or the promotion of fetal growth; to regulate neurotransmission or neural growth; to reduce or inhibit allergic/inflammatory reactions due to mast cell degranulation or involvement of immune response elements, such as macrophages; to inhibit bacterial and fungal growth; to facilitate repair and/or healing of skin chafing, skin lesions or wounds; and to promote bone and organ development. Such important uses were first taught and described by applicants in the instant application.

The invention as presently claimed is regarded to enjoy inventive step in view of the complete teachings of D1, which discloses that adrenomedullin or pro-AM-N20 are participants in blood pressure control. D1 also shows the tissue distribution of

both adrenomedullin and proAM-N20 (Table 1, page 13, and Table 2, page 17), wherein the highest amount of these peptides is found in human PC and adrenal medulla, with a small amount in lung and very little in brain, intestine and ventricle. D1 contains no teaching or suggestion that the isolated AM peptides as presently claimed can be used, for example, to regulate or control cancer cell proliferation, or in the regulation of blood glucose levels, or to regulate mast cell degranulation or immune responses related to inflammation. The low levels of adrenomedullin reported by D1 in brain cortex can be considered to be a teaching away from the findings of applicants.

The disclosure of D1 does not lead one having skill in the pertinent art to isolate and employ the AM peptides described by applicants in a variety of novel therapeutic and prophylactic uses. The use of the specified AM peptides for such utilities and activities was newly discovered by applicants and disclosed in the present specification. Thus, it is submitted that the activities and utilities of the AM peptides described in the present application are inventive and could not have been deduced in an obvious manner from the teachings of D1. D1 provides no teaching to the reader to select and isolate the AM peptides used by applicants; it provides no teaching to use the claimed peptides for the newly-discovered activities disclosed by applicants in the present application.

In view of the foregoing, applicants believe that the requirements of inventive step of the presently claimed invention have been met. Contrary to the unsubstantiated conclusion on sheet four, paragraph 3, of the Written Opinion, it is

submitted that "the activity mentioned in D1" and the described novel activities and uses of the AM peptides disclosed and claimed by applicants do not "respond to the same physiological system or describe different effects of the same system". Neither D1 nor any cited prior art reference teaches or suggests that the effects described in D1 (i.e., "vasorelactant activity, catecholamine secretion inhibitory effect and Na channel inhibitory effect") are effective in diseases other than those described in D1, namely, cardiac diseases, cardiac infarction and hypertension. Accordingly, applicants' claimed invention could not have been deduced in an obvious manner from the distinctly different and specific teachings of D1.

Moreover, neither D2, which teaches that the entire 52 amino acid long adrenomedullin has varied tissue distribution, nor D3, which teaches a different adrenomedullin peptide as but one type of biologically active polypeptide containing conformation-constraining moieties, either alone or together, provides teachings that makes obvious applicants' invention as presently claimed.

To meet the requirements of Rule 5.1(a)(ii) PCT, document D1 has been identified in the description of the above-identified application and the relevant background art disclosed therein briefly discussed at enclosed replacement pages 3/1, lines 33-35 and page 3/2, line 1.

Applicants provide herewith replacement pages 3/1, 3/2, 8/1, 12/1, 13/1 and 15/1 of the specification to correct oversights noticed upon review of the application at the time of responding to the Written Opinion.

Specifically, on replacement page 3/1, lines 33-35, and page 3/2, lines 1-2,

reference to document D1 is made in the Background section of the application. On page 8/1, Figure 8 and the description thereof have been amended to read "Figures 8A and 8B" in accordance with the corrected drawings. On replacement page 12/1, Figure 25 and the description thereof have been amended to read "Figures 25A and 25B" in accordance with the corrected drawings. On replacement page 13/1, the phrase "novel AM peptide" directly above the table has been replaced with -- novel isolated AM peptides and oligonucleotide sequences --. On replacement page 15/1, line 28, "in" before "*in situ*" has been changed to -- and --.

In view of the replacement claims and the explanation presented above, it is submitted that the present claims are acceptable for international examination and enjoy inventive step and novelty in view of the cited prior art.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: August 21, 1997

By: Leslie A. Serunian
Leslie A. Serunian
Agent for Applicant

Mailing Address:

MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154 USA
(212) 758-4800 Telephone
(212) 751-6849 Facsimile

What is claimed is:

1. An isolated adrenomedullin peptide selected from the group consisting of PO70 (SEQ ID NO: 1), PO71 (SEQ ID NO: 2) and PO72 (SEQ ID NO: 3).

2. An antibody reactive with at least one of the peptides of claim 1.

3. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for use in treating a patient suffering from a cancer or tumor, comprising contacting cancer or tumor cells with an amount of said medicament effective to prevent or treat the cancer or tumor.

4. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith for the treatment of a patient suffering from a cancer or tumor, comprising contacting cancer or tumor cells with an amount of said peptides or antibodies effective to prevent or treat the cancer or tumor.

5. Use according to claims 3 or 4 wherein the cancer or tumor cells are selected from the group consisting of adrenal, nervous system, lung, colon, ovarian, prostate, chondrosarcoma, pancreas, or breast.

6. A method for diagnosing or monitoring a disease other than hypotension, hypertension or cardiac incompetence, comprising measuring the levels of adrenomedullin in a sample, wherein the presence or absence of adrenomedullin indicates the existence of, or predisposition to, the disease.

7. The method of claim 6, wherein the disease is diabetes, renal disease, bone disease, skin disease, or hematopoietic cell disease.

8. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for use in preventing or treating a patient suffering from type II diabetes, comprising providing to the patient an amount of said medicament effective to regulate insulin secretion and blood glucose metabolism.

9. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith for the treatment of a patient suffering from type II diabetes, comprising providing to the patient an amount of said peptides or antibodies effective to regulate insulin secretion and blood glucose metabolism.

10. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for use in diagnosing or treating women for conditions related to pregnancy.

11. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith for use in diagnosing or treating women for conditions related to pregnancy.

12. Use according to claim 10 or claim 11 wherein the condition is preeclampsia or fetal growth.

13. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for use in regulating neuronal activity in areas of the central nervous system, comprising administering to a subject said medicament in an

amount effective to regulate neurotransmission or neuron growth.

14. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith for use in regulating neuronal activity in areas of the central nervous system, comprising administering to a subject said peptides or antibodies in an amount effective to regulate neurotransmission or neuron growth.

15. Use of the adrenomedullin antibodies of claim 2 in the manufacture of a medicament for use in regulating, lessening, or inhibiting an allergic or inflammatory response due to the degranulation of mast cells or involvement of immune response cells, comprising administering said medicament in an amount effective to lessen or inhibit the degranulation of mast cells.

16. Use of the adrenomedullin antibodies of claim 2 for use in regulating, lessening, or inhibiting an allergic or inflammatory response due to the degranulation of mast cells or involvement of immune response cells, comprising administering said antibodies in an amount effective to lessen or inhibit the degranulation of mast cells.

17. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for treating bacterial or fungal infections, comprising administering to a subject said medicament in an amount effective to inhibit or prevent bacterial or fungal growth.

18. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith for treating bacterial or fungal infections, comprising administering to a subject said peptides or antibodies in an amount effective to inhibit or prevent bacterial or fungal growth.

19. Use of the adrenomedullin peptides of claim 1 in the manufacture of a medicament for use in facilitating the repair or healing of chafed skin, skin lesions, wounds, and surgical incisions, comprising applying said medicament to the surface of the skin of a subject in an amount effective to facilitate the repair or healing.

20. Use of the adrenomedullin peptides of claim 1 for facilitating the repair or healing of chafed skin, skin lesions, wounds, and surgical incisions, comprising applying said adrenomedullin peptides to the surface of the skin of a subject in an amount effective to facilitate the repair or healing.

21. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in the manufacture of a medicament for use in promoting organ and bone development.

22. Use of the adrenomedullin peptides of claim 1 or antibodies reactive therewith in promoting organ and bone development.

23. A pharmaceutical composition comprising the adrenomedullin peptides of claim 1.

24. A pharmaceutical composition comprising the adrenomedullin antibodies of claim 2.

25. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for use in treating a patient suffering from a cancer or tumor, comprising contacting cancer or tumor cells with an amount of said medicament effective to prevent or treat the cancer or tumor.

26. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for the treatment of a patient suffering from a cancer or tumor, comprising contacting cancer or tumor cells with an amount of said peptide or antibodies effective to prevent or treat the cancer or tumor.

27. Use according to claims 25 or 26 wherein the cancerous cells are selected from the group consisting of adrenal, nervous system, lung, colon, ovarian, prostate, chondrosarcoma, pancreas, or breast.

28. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith

in the manufacture of a medicament for use in preventing or treating a patient suffering from type II diabetes, comprising providing to the patient an amount of said medicament effective to regulate insulin secretion and blood glucose metabolism.

29. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for the treatment of a patient suffering from type II diabetes, comprising providing to the patient an amount of said peptides or antibodies effective to regulate insulin secretion and blood glucose metabolism.

30. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for use in diagnosing or treating women for conditions related to pregnancy.

31. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for use in diagnosing or treating women for conditions related to pregnancy.

32. Use according to claim 30 or claim 31 wherein the condition is preeclampsia or fetal growth.

33. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for use in regulating neuronal activity in areas of the central nervous system, comprising administering to a subject said

medicament in an amount effective to regulate neurotransmission or neuron growth.

34. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for use in regulating neuronal activity in areas of the central nervous system, comprising administering to a subject said peptide or antibodies in an amount effective to regulate neurotransmission or neuron growth.

35. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for use in regulating, lessening, or inhibiting the allergic response due to the degranulation of mast cells, comprising administering said medicament in an amount effective to lessen or inhibit the degranulation of mast cells.

36. Use of antibodies reactive with isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) for regulating, lessening, or inhibiting the allergic response due to the degranulation of mast cells, comprising administering said antibodies in an amount effective to lessen or inhibit the degranulation of mast cells.

37. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for treating bacterial or fungal infections, comprising administering to a subject said medicament in an amount effective to inhibit or prevent bacterial or fungal growth.

38. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for treating bacterial or fungal infections, comprising

administering to a subject said peptide or antibodies in an amount effective to inhibit or prevent bacterial or fungal growth.

39. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) in the manufacture of a medicament for use in facilitating the repair or healing of chafed skin, skin lesions, wounds, and surgical incisions, comprising applying said medicament to the surface of the skin of a subject in an amount effective to facilitate the repair or healing.

40. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) for facilitating the repair or healing of chafed skin, skin lesions, wounds, and surgical incisions, comprising applying said adrenomedullin peptides to the surface of the skin of a subject in an amount effective to facilitate the repair or healing.

41. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith in the manufacture of a medicament for use in promoting organ and bone development.

42. Use of isolated adrenomedullin peptide PAMP-20 (SEQ ID NO: 7) or antibodies reactive therewith for promoting organ and bone development.

43. An isolated adrenomedullin oligonucleotide selected from the group consisting of AM_{94-114} (SEQ ID NO: 4), $AM_{444-464}$ (SEQ ID NO: 5) and $AM_{289-309}$ (SEQ ID NO: 6).

(1994); T. A. Murphy and W. K. Samson, Endocrinology 136, 2459 (1995); T. Yamaguchi, K. Baba, Y. Doi, K. Yano, Life Sci. 56, 379 (1995); W. K. Samson, T. Murphy, D. A. Schell, Endocrinology 136, 2349 (1995)). Finally, AM has been shown to be expressed in a variety of human tumors of both neural and pulmonary lineage including ganglioblastoma/neuroblastoma (F. Satoh, et al., J. Clin. Endocrinol. Metabol. 80, 1750 (1995)), small cell lung cancer, adenocarcinoma, bronchoalveolar carcinoma, squamous cell carcinoma, and lung carcinoids (Martínez, et al., Endocrinology 136, 4099 (1995)). In an attempt to further study the distribution of AM in human tumors and determine its role in these malignant disorders, we used molecular, biochemical and in vitro techniques to analyze 59 human cancer cell lines from solid tumors and hemopoietic lineage.

AM's role as a vasodilatory agent has been extensively studied (C. Nuki et al., Biochem. Biophys. Res. Commun. 196, 245 (1993); Q. Hao et al., Life Sci. 54, 265 (1994); D. Y. Cheng et al., Life Sci., 55, 251 (1994); C. J. Feng, B. Kang, A. D. Kaye, P. J. Kadowitz, B. D. Nossaman, Life Sci., 433 (1994)). It acts through specific receptors in the plasma membrane to activate adenylate cyclase activity and modulate $Ca^{2}+$ flux in the target cells (S. Eguchi et al., Endocrinology 135, 2454 (1994); Y. Shimekake et al., J. Biol. Chem. 270, 4412 (1995)). These signal transduction pathways are involved in numerous physiological processes, including the regulation of hormone secretion. It is well established that regulation of intracellular cAMP modulates hormone release in the pancreas (Y. Korman, S. J. Bhathena, N. R. Voyles, H. K. Oie, L. Recant, Diabetes 34, 717 (1985); C. B. Wollheim, Diabetes 29, 74 (1980)). AM has also been reported to have an effect on Na^{+} channel activity (EP Application No. 0 622 458 A2). Since AM has been reported to influence the secretion rate of several

Neurochem. 64, 459 (1995); EP Application No. 0 622 458 A2), adrenocorticotropin (W. K.

at lower magnification (x120), after application of the in situ RT-PCR technique (Figure 5C). Arrows point to blood vessels whose endothelial layers are clearly positive. Omission of primers in the PCR mixture gave negative staining (Figure 5D).

Figures 6A and 6B: Figure 6A and 6B set forth photographs of the detail of chondrocytes immunostained with anti-AM (Figure 6A) and with the antiserum preabsorbed with P072 (Figure 6B). (Magnification x700).

Figure 7A and 7B: Figures 7A and 7B set forth photographs of alveolar macrophages labeled for AM mRNA after in situ RT-PCR (Figure 7A) and negative control without reverse transcriptase (Figure 7B). (Magnification x450).

Figure 8A and 8B: Characterization of AM by RT-PCR in mRNA from normal tissues and pulmonary tumor cell lines. The PCR products had the proper size (410bp) when visualized with UV light (Figure 8B), and they hybridized with the specific probe after Southern blot (Figure 8A). H146 and H345 are small cell carcinomas, H676 is an adenocarcinoma, H720 is a carcinoid, and H820 is a bronchioalveolar carcinoma. H146 was the only cell line that tested negative for AM.

Figures 9A and 9B: Figures 9A and 9B set forth photographs of cell line H820 (bronchioalveolar carcinoma) showing a cytoplasmic distribution of AM mRNA, as revealed by in situ RT-PCR (Figure 9A), and a serial section demonstrating that the staining disappears when the reverse transcription step is omitted (Figure 9B). (Magnification x550)

Figures 10A and 10B: Figures 10A and 10B set forth

Figures 23A and 23B: Figures 23A and 23B set forth the antimicrobial activity of AM and PAMP.

Figure 24: Figure 24 indicates the effect of AM on the germination of C. albicans.

Figures 25A and 25B: Figures 25A and 25B set forth the distribution of amphipathic regions for AM and PAMP as calculated for a-helix/b-sheet angle parameters (Eisenberg), and the helical wheel projection display for AM and PAMP (DNASTAR).

Figures 26A-26D: Figure 26 sets forth a representative sample of human tumor cell lines and normal human tissues screened for AM and AM-R. Southern blot analysis demonstrates the predicted 410 bp product for AM **(A)** and a 471 bp product for AM-R mRNA **(B)** after RT-PCR amplification. **(C)** Western blot analysis of cell extracts shows immunoreactive species of 18, 14, and 6 kDa when using a rabbit antiserum to AM. In addition, there is a 22 kDa immunoreactive entity that may be attributed to post-translational processing. **(D)** The absorption control was negative.

Figures 27A-27D: Figure 27 sets forth the immunohistochemical and in situ RT-PCR analysis of human cancer cell lines for AM. **(A)** Immunohistochemical analysis for AM in SCLC H774 and **(B)** ovarian carcinoma cell line NIH: Ovcar-3. Note the peripheral distribution of AM immunoreactivity in H774 colonies. **(C)** In situ RT-PCR for AM mRNA in carcinoid cell line H720 and **(D)** negative control in a serial section where primers were substituted by water in the PCR mixture.

Figures 28A and 28B: Figure 28 sets forth the growth effects of AM. A representative human tumor cell line,

MCF-7, was used to show the growth effects, receptor binding and cAMP variation by AM under serum-free, hormone-free conditions. **(A)** Inhibitory effects of MoAb-G6 (●) compared with no effect from its mouse myeloma isotypic control, IgAκ (○). **(B)** Effects of MoAb-G6 were overcome by the addition of synthetic AM (○) compared with the addition of AM alone (●). **(C)** Specific receptor binding is measurable for AM (○) while it is negligible for PAMP (●) or P072 (□). **(D)** Cyclic AMP is increased with the addition of synthetic AM in a dose-dependent manner.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally provides novel adrenomedullin (AM) peptides and AM antibodies, pharmaceutical compositions comprising said peptides and antibodies, and their use as pharmaceutically active agents.

Specifically, the present invention relates to the following novel isolated AM peptides and oligonucleotide sequences:

PO70 (YY-PreproAM$_{34-41}$)	Y-Y-W-N-K-W-A-L-S-R-NH_2 (SEQ. ID. NO. 1)
PO71 (YGG-PreproAM$_{122-131}$)	Y-G-G-H-Q-I-Y-Q-F-T-D-K-D-NH_2 (SEQ. ID. NO. 2)
PO72 (PreproAM$_{116-146}$)	T-V-Q-K-L-A-H-Q-I-Y-Q-F-T-D-K-D-K-D-N-V-A-P-R-S-K-I-S-P-Q-G-Y-NH_2 (SEQ. ID. NO. 3)
Sense primer (AM 94-114)	5′-AAG-AAG-TGG-AAT-AAG-TGG-GCT-3′ (SEQ. ID. NO. 4)
Antisense primer (AM 444-464)	5′-TGG-CTT-AGA-AGA-CAC-CAG-AGT-3′ (SEQ. ID. NO. 5)
Antisense probe (AM 289-309)	5′-CTG-GAA-GTT-GTT-CAT-GCT-CTG-3′ (SEQ. ID. NO. 6)
Proadrenomedullin N-terminal 20 peptide (PAMP-20)	A-R-L-D-V-A-S-E-F-R-K-K-W-N-K-W-A-L-S-R-NH_2 (SEQ. ID. NO. 7)

bronchial epithelium are supposed to be restricted to the mechanical transport of the mucous layer through ciliary beat, but other regulatory peptides, such as endothelins, have been described in this cell type (Giaid, et al., Am J Respir Cell Mol Biol 4:50-56 (1990)). The expression of AM in the lining epithelium and macrophages points to a possible protective action against pathogens, similar to that observed for other peptides, such as the magainins present in the airway epithelium (Diamond, et al., Proc Natl Acad Sci USA 90:4596-4600 (1993)) or the tracheal antimicrobial peptide (Diamond, et al., Proc Natl Acad Sci USA 88:3952-3956 (1991)).

The distribution of AM in normal lung described herein colocalizes with the pattern previously reported for the peptide-amidating enzymes (Saldise, et al., J. Histochem. Cytochem. 1996). In this reference, it was postulated the potential existence of yet unknown amidated peptides concomitantly expressed at the same sites of enzyme production. The studies herein suggest that AM may be one of those predicted peptides. It has been demonstrated that amidation is important for AM activity; the amidated form of AM has 50 times greater affinity for the receptor than the nonamidated form (Eguchi, et al., Endocrinology 135:2454-2458 (1994)).

To characterize the functions of AM in normal tissues, the distribution of AM was studied in normal and malignant lung using immunocytochemical techniques to localize the peptide and in situ reverse transcriptase-polymerase chain reaction (RT-PCR) to study the expression of its messenger RNA (mRNA) in formalin-fixed paraffin-embedded specimens.

(2) Human AM/AM-R mRNA expression in normal and malignant cells

RT-PCR was used to evaluate AM ligand and receptor mRNA in a variety of cancer cell lines of